

## ORIGINAL ARTICLE

# Is the humoral immunity dispensable for the pathogenesis of psoriasis?

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## Abstract

**Background** Imbalances of T-cell subsets are hallmarks of disease-specific inflammation in psoriasis. However, the relevance of B cells for psoriasis remains poorly investigated.

**Objective** To analyse the role of B cells and immunoglobulins for the disease-specific immunology of psoriasis.

**Methods** We characterized B-cell subsets and immunoglobulin levels in untreated psoriasis patients ( $n = 37$ ) and compared them to healthy controls ( $n = 20$ ) as well as to psoriasis patients under disease-controlling systemic treatment ( $n = 28$ ). B-cell subsets were analysed following the flow cytometric gating strategy based on the surface markers CD24, CD38 and CD138. Moreover, immunofluorescence stainings were used to detect IgA in psoriatic skin.

**Results** We found significantly increased levels of IgA in the serum of treatment-naïve psoriasis patients correlating with disease score. However, IgA was only observed in dermal vessels of skin sections. Concerning B-cell subsets, we only found a moderately positive correlation of CD138<sup>+</sup> plasma cells with IgA levels and disease score in treatment-naïve psoriasis patients. Confirming our hypothesis that psoriasis can develop in the absence of functional humoral immunity, we investigated a patient who suffered concomitantly from both psoriasis and a hereditary common variable immune defect (CVID) characterized by a lack of B cells and immunoglobulins. We detected variants in three of the 13 described genes of CVID and a so far undescribed variant in the ligand of the TNFRSF13B receptor leading to disturbed B-cell maturation and antibody production. However, this patient showed typical psoriasis regarding clinical presentation, histology or T-cell infiltrate. Finally, in a group of psoriasis patients under systemic treatment, neither did IgA levels drop nor did plasma cells correlate with IgA levels and disease score.

**Conclusion** B-cell alterations might rather be an epiphenomenal finding in psoriasis with a clear dominance of T cells over shifts in B-cell subsets.

Received: 7 February 2018; Accepted: 23 May 2018

## Conflict of interest

None declared.

## Funding sources

Helmholtz Association, German Research Foundation Deutsche Forschungsgemeinschaft, European Research Council.

## Introduction

Psoriasis is a highly prevalent chronic inflammatory skin disease, which is associated with metabolic and cardiovascular comorbidities.<sup>1–3</sup> In the pathogenesis of psoriasis, T lymphocytes, in particular Th1 and Th17 cells,<sup>4</sup> and their cytokines have been identified as key cellular players leading to a broad set of effective and specific therapies.<sup>5,6</sup> However, increasing evidence from

other Th17-mediated diseases such as multiple sclerosis and rheumatoid arthritis suggests that B cells might be equally important players.<sup>7</sup> In line with this, clinical trials demonstrated that B-cell depleting therapies reduced relapses and disease activity in patients suffering from multiple sclerosis and improved disease symptoms in patients with rheumatoid arthritis.<sup>7–10</sup>

As precursors of differentiated plasma cells, B lymphocytes not only produce antibodies, but also play an important immunological role as antigen-presenting cells and producers of

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a broad array of cytokines, which control early and established T-cell-mediated responses.<sup>11</sup>

In psoriasis, B cells were indeed detected in lesional skin,<sup>12,13</sup> and altered frequencies of circulating B lymphocytes in psoriasis have been described.<sup>14</sup> However, it is still unclear whether B cells have a functional role in the disease-specific immunology of psoriasis or whether alterations only occur as a bystander phenomenon. In-depth data – in particular comprehensive characterization of different B-cell phenotypes in the context of their developmental stage – are sparse. Recently, the subset of CD19<sup>+</sup>CD24<sup>hi</sup>CD38<sup>hi</sup> B cells has been shown to maintain regulatory T cells while limiting Th1 and Th17 differentiation,<sup>15</sup> which may imply significant impact in the context of the psoriatic inflammation.

In this study, B-cell subtypes and immunoglobulins were analysed in peripheral blood in a large cohort of psoriasis patients as compared to healthy controls. Our findings were tested for correlation to disease activity (PASI) and validated by immunofluorescence stainings. Furthermore, to estimate the significance of B cells in psoriatic inflammation *in vivo*, a patient suffering from psoriasis as well as a B-cell immunodeficiency was investigated intensively. Finally, B-cell subsets were compared between the group of therapy-naïve and systemically treated psoriasis patients to detect whether disease control is dependent on B-cell alterations.

## Materials and methods

### Patients

Thirty-seven treatment-naïve patients with psoriasis and Psoriasis Area and Severity Index [PASI] score > 5 (42.0 ± 2.4 years; PASI: range 6.2–42.3, mean 14.9), and 20 healthy volunteers (48.2 ± 3.2 years) were enrolled in this study. No age ( $P = 0.15$ , one-way ANOVA) or sex ( $P = 0.38$ , chi-square test) disparities were observed between the groups. To define whether successful systemic treatment would affect B-cell and immunoglobulin status, a second cohort of 28 patients with psoriasis under systemic treatment with PASI <5 (52.1 ± 2.1 years; PASI: range 0.0–4.7, mean 1.7) was established. Among these patients, eight patients were treated with anti-TNF- $\alpha$  medication, eight patients with methotrexate, five patients with anti-IL-23/IL-12 medication, three patients with fumaric acid esters, three patients with PDE-4 inhibitor and one patient with acitretin. Severity scores were obtained using the PASI system. All patients and control subjects gave their written consent to participate in the study, and the study was approved by the local ethical committee (project number 44/16S).

### Whole blood cell surface staining

Whole peripheral blood was lysed using red blood cell lysis buffer containing NH<sub>4</sub>Cl, KHCO<sub>3</sub> and EDTA dissolved in H<sub>2</sub>O. After blocking unspecific antibody binding with Human BD Fc

Block (Becton, Dickinson and Company, Franklin Lakes, NJ), peripheral blood cells were stained using fluorochrome-labelled antibodies binding to cell surface markers (anti-CD19, anti-CD24, anti-CD38, anti-CD138, anti-CD3; all BD; anti-CD138: R&D Systems, Minneapolis, MN; anti-CD20, anti-IgA: Miltenyi Biotec, Bergisch Gladbach, Germany). Dead cells were detected and excluded from analysis by LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit (Thermo Fisher Scientific, Waltham, MA).

### Flow cytometric analysis and nomenclature

The CD19<sup>+</sup> B cells were grouped in five subsets according to their CD24 and CD38 expression, two markers that are expressed on all B cells but differentially regulated during B-cell development.<sup>16</sup> This well-established approach enables to distinguish CD24<sup>+</sup>CD38<sup>-</sup> primarily memory B cells, CD24<sup>int</sup>CD38<sup>int</sup> mature naïve B cells, CD24<sup>hi</sup>CD38<sup>hi</sup> transitional/regulatory B cells, CD24<sup>-</sup>CD38<sup>+</sup> plasmablasts/plasma cells and CD24<sup>-</sup>CD38<sup>-</sup> new memory B cells.<sup>17–19</sup> Furthermore, we analysed CD138 expression to gain deeper insight into the plasma cell compartment.

Cells stained with fluorochrome-conjugated antibodies against corresponding surface antigens were acquired with a LSRFortessa flow cytometer (BD) and analysed using FlowJo Software, version 10 (FlowJo, LLC, Ashland, OR).

### Statistical analysis

Statistical analysis was performed using GraphPad Prism 7.00 Software (GraphPad Software, La Jolla, CA). Mann–Whitney *U*-test was used to compare between variables. Variables were correlated using Spearman rank correlation. All values are expressed as means with standard error of the mean (SEM).

### Ethics

All methods were carried out in accordance with relevant guidelines and regulations. Human patient procedures were approved by the Ethics Committee of the Technical University of Munich (project number 44/16S), and all patients and control subjects provided informed consent.

## Results

### IgA serum levels are elevated in psoriasis and correlated with disease severity

In untreated patients with psoriasis, as well as in our cohort of control subjects, we first analysed humoral serum proteins, namely IgM, IgA, IgG, IgE and complement factors C3 and C4. We found that C3 and C4 concentrations were higher in the serum of psoriasis patients (125 ± 4.6 mg/dL for C3; 26.2 ± 1.2 mg/dL for C4) than in healthy controls (105.7 ± 3.3 mg/dL for C3,  $P = 0.0034$ ; 21.4 ± 1.3 mg/dL for C4,  $P = 0.0096$ , Figure S1A,B, Supporting Information). Concerning immunoglobulins, we found no differences in the serum

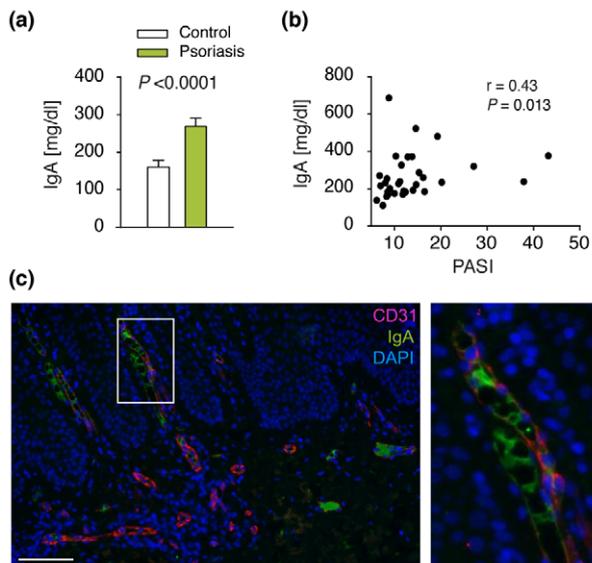
levels of IgG and IgM between the cohorts (Figure S1C,D, Supporting Information). IgE levels were significantly increased in psoriasis ( $256.8 \pm 62.2$  IU/mL) compared to healthy controls ( $78.39 \pm 24.61$  IU/mL,  $P = 0.0146$ ; Figure S1E, Supporting Information). Most significantly, however, we found IgA levels higher in psoriasis ( $269.2 \pm 20.6$  mg/dL) than in healthy controls ( $160.54 \pm 17.63$  mg/dL,  $P < 0.0001$ , Fig. 1a). More importantly, IgA serum levels but not IgE levels in the serum of psoriasis patients showed a correlation with PASI ( $r = 0.43$ ,  $P = 0.013$  for IgA,  $r = 0.23$ ,  $P = 0.21$  for IgE; Fig. 1b, Figure S1F, Supporting Information). Accordingly, IgA could also be detected in psoriatic skin. However, in all sections ( $n = 18$ ) examined, IgA was only found in dermal vessels of lesional psoriatic skin and not in epidermal or dermal tissue (Fig. 1c).

### Characterization of B-cell subsets does not reveal profound psoriasis-specific aberrations

Further investigating the discrepancy of elevated IgA in serum of psoriasis patients but absent accumulation in psoriatic skin, we analysed B cells as sources of IgA in both psoriatic skin and blood. In line with our findings for IgA in the skin, immunofluorescence double stainings of both CD19 and CD8 (marker for

cytotoxic T cells) revealed only few B cells as compared to the abundance of T cells in psoriatic skin (Figure S4, Supporting Information). To clarify whether elevated IgA serum levels are paralleled by an altered composition of B-cell subsets in the peripheral blood, we next analysed the distinct B-cell subsets using the common gating strategy based on CD24 and CD38.<sup>17,18</sup> Within the five different subsets in the CD24/CD38 gates, no significant differences between the two groups of untreated psoriasis patients and control subjects were found for the CD19<sup>+</sup>CD38<sup>-</sup>CD24<sup>-</sup> new memory B cells (psoriasis:  $2.2 \pm 0.2\%$ , control subjects:  $2.1 \pm 0.3$ ,  $P = 0.7662$ ), CD19<sup>+</sup>CD38<sup>hi</sup>CD24<sup>hi</sup> transitional regulatory B cells (psoriasis:  $4.2 \pm 0.4\%$ , control subjects:  $5.05 \pm 0.7\%$ ,  $P = 0.7781$ ), CD19<sup>+</sup>CD38<sup>-</sup>CD24<sup>hi</sup> primarily memory B cells (psoriasis:  $27 \pm 2.6\%$ , control subjects:  $24.4 \pm 2.8\%$ ,  $P = 0.8316$ ) and mature B cells, as defined by CD19<sup>+</sup>CD38<sup>int</sup>CD24<sup>int</sup> (psoriasis:  $59.9 \pm 2.7\%$ , control subjects:  $62.6 \pm 2.8\%$ ,  $P = 0.8386$ ). Regarding the population of CD19<sup>+</sup>CD24<sup>-</sup>CD38<sup>+</sup> plasmablasts, a trend for a higher proportion was observed in psoriasis ( $2 \pm 0.4\%$ ) as compared to control subjects ( $1.4 \pm 0.3\%$ ,  $P = 0.2561$ , Fig. 2a).

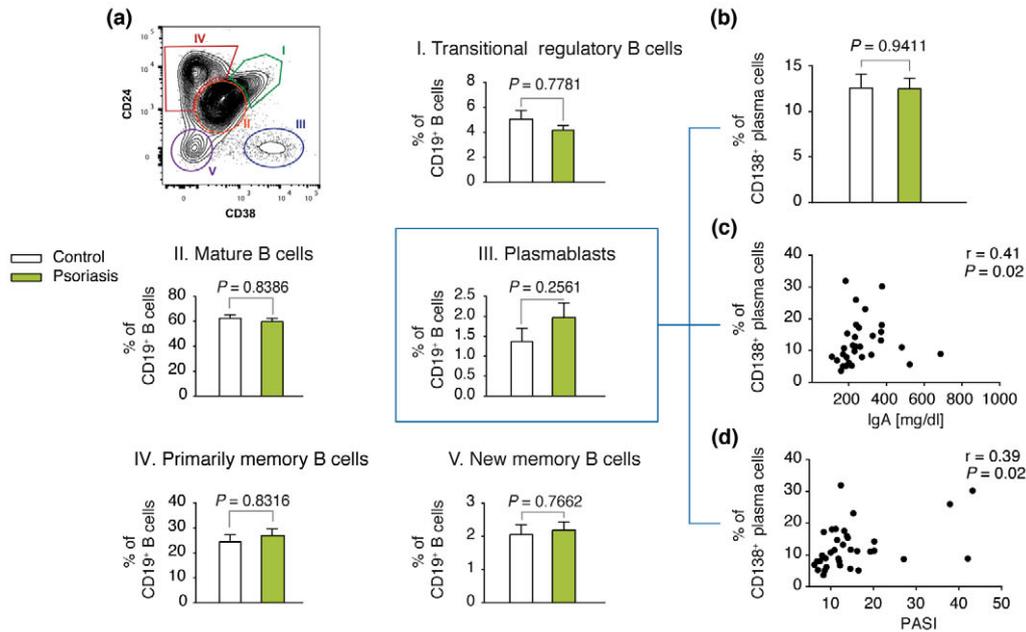
A substantial proportion of the cells within the fraction of CD19<sup>+</sup>CD24<sup>-</sup>CD38<sup>+</sup> plasmablasts in the blood were previously described to express CD138, a proteoglycan that is a hallmark of plasma cells.<sup>20</sup> Moreover, as steady-state circulating CD38<sup>hi</sup>CD138<sup>+</sup> plasma cells are known to secrete IgA<sup>20</sup> and IgA was elevated in our cohort of psoriasis patients as compared to healthy controls, we next focused on the fraction of CD19<sup>+</sup>CD24<sup>-</sup>CD38<sup>+</sup>CD138<sup>+</sup> plasma cells within the population of CD19<sup>+</sup>CD24<sup>-</sup>CD38<sup>+</sup> plasmablasts. Although similar frequencies of this cell population were detected in psoriasis ( $12.5 \pm 1.18\%$ ) and healthy volunteers ( $12.6 \pm 1.5$ ,  $P = 0.9411$ , Fig. 2b), we found only in psoriasis but not in healthy controls a moderate correlation between IgA levels and the CD19<sup>+</sup>CD24<sup>-</sup>CD38<sup>+</sup>CD138<sup>+</sup> subset ( $r = 0.4$ ,  $P = 0.02$  for psoriasis;  $r = -0.03$ , Fig. 2c;  $P = 0.9$  for healthy controls). Besides, PASI was positively correlated with the frequency of CD19<sup>+</sup>CD24<sup>-</sup>CD38<sup>+</sup>CD138<sup>+</sup> plasma cells ( $r = 0.39$ ;  $P = 0.02$ , Fig. 2d). Furthermore, we directly examined IgA expression on CD19<sup>+</sup>CD24<sup>-</sup>CD38<sup>+</sup>CD138<sup>+</sup> plasma cells and found that CD19<sup>+</sup>CD24<sup>-</sup>CD38<sup>+</sup>CD138<sup>+</sup> cells are indeed IgA-positive. Moreover, CD19<sup>+</sup>CD24<sup>-</sup>CD38<sup>+</sup>CD138<sup>+</sup>IgA<sup>+</sup> plasma cell frequencies are also correlated with PASI ( $r = 0.883$ ,  $P = 0.015$ , Figure S5A,B, Supporting Information).



**Figure 1** IgA serum levels are elevated in psoriasis and correlated with disease severity. IgA levels are significantly increased in psoriasis as compared to healthy controls (a). In psoriasis patients, IgA levels show a moderate correlation with disease score PASI (b). Representative immunofluorescence staining of psoriatic skin shows that IgA could also be detected in psoriatic skin (c). However, IgA was only found in dermal vessels of lesional psoriatic skin and not in epidermal or dermal tissue. Scale bar = 100  $\mu$ m. Inset at 3 $\times$  magnification.

### Development of psoriasis in a patient suffering from common variable immune deficiency accompanied by a severe lack of B cells and immunoglobulins

To interpret our results for the relevance of disease pathogenesis in human psoriasis, we eventually had the chance to also analyse a patient (A.B.) who suffered from a hereditary common variable immunodeficiency characterized by a lack of B cells and



**Figure 2** Characterization of B-cell subsets using the CD24/CD38 gating strategy. Within the five different subsets in the CD24/CD38 gates, no significant differences between the two groups of untreated psoriasis patients and control subjects were found for the CD19<sup>+</sup>CD38<sup>hi</sup>CD24<sup>hi</sup> transitional regulatory B cells (I), CD19<sup>+</sup>CD38<sup>int</sup>CD24<sup>int</sup> mature B cells (II), CD19<sup>+</sup>CD38<sup>-</sup>CD24<sup>hi</sup> primarily memory B cells (IV) and CD19<sup>+</sup>CD38<sup>-</sup>CD24<sup>-</sup> new memory B cells (V). Regarding the population of CD19<sup>+</sup>CD24<sup>-</sup>CD38<sup>+</sup> plasmablasts (III), a trend for a higher proportion was observed in psoriasis (a). Regarding the fraction of CD19<sup>+</sup>CD24<sup>-</sup>CD38<sup>+</sup>CD138<sup>+</sup> plasma cells within the fraction of plasmablasts, similar frequencies were detected in psoriasis and healthy volunteers (b), but a moderate correlation between plasma cells with IgA levels (c) and PASI (d) was found.

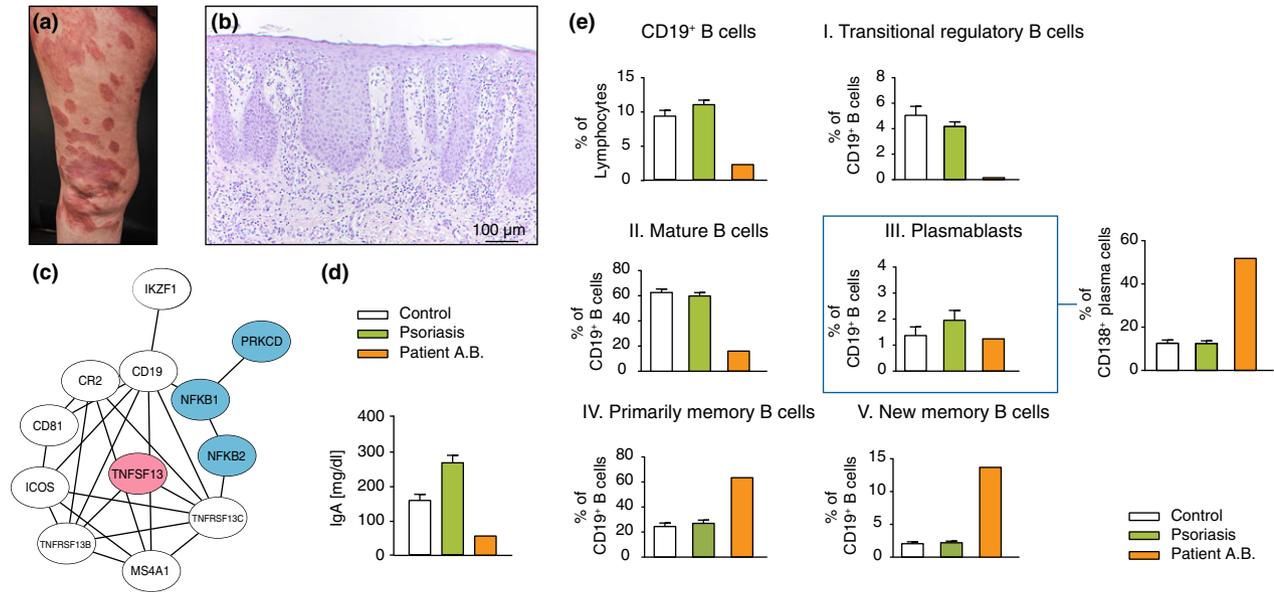
immunoglobulins and – at the age of thirty – developed psoriasis for the first time. On admission, the patient displayed the full phenotype of psoriasis vulgaris both clinically (Fig. 3a) and histologically (Fig. 3b). The family history for psoriasis was positive, and the PASI of A.B. on admission was 30. In respect of the findings from the cohort of psoriasis patients, this specific patient might offer the opportunity to clarify the question whether B cells are of high relevance in the pathogenesis of psoriasis or – in contrary – whether the lack of B cells had been irrelevant for the development of psoriasis. We first analysed the functionality of peripheral blood mononuclear cells (PBMCs) and found that PBMC stimulated by  $\alpha$ -CD3 and  $\alpha$ -CD28, as well as by phytohemagglutinin (PHA) and pertussis toxin, showed substantial proliferation as assessed by H3-thymidine incorporation indicating intact T-cell function upon stimulation (Figure S2A, Supporting Information). Next, we isolated T cells from lesional skin of the patient, stimulated them and compared their cytokine profile to a representative cohort of cytokine profiles from patients with psoriasis randomly picked from the untreated cohort ( $n = 11$ ). We found comparative values for A.B. and psoriasis patients with high amounts of TNF- $\alpha$ , IFN- $\gamma$  and IL-17 indicating a Th17 profile that was not altered by a lack

of B cells and their respective cytokines (Figure S2B, Supporting Information).

To characterize the underlying immune defect of A.B. in more detail, we performed total RNA sequencing of lesional skin in this patient and analysed genetic variants within these transcripts. In the transcripts of the 13 described CVID genes, we identified six synonymous variants and one non-synonymous variant in three of them, namely NFKB1, NFKB2 and PRKCD. In addition, we identified one non-synonymous variant in TNFSF13, the ligand of the TNFRSF13B receptor. This receptor also belongs to the 13 described genes of CVID and shows strong integration with the other CVID genes when depicting all CVID genes in a network (Fig. 3c).

We then analysed peripheral B-cell subsets from PBMCs and serum IgA in A.B. We measured a strong decrease of IgA levels in A.B. (57 mg/dL) as compared to psoriasis ( $269.2 \pm 20.6$  mg/dL) and healthy controls ( $160.54 \pm 17.63$  mg/dL, Fig. 3d). Besides, we found a marked reduction in peripheral CD19<sup>+</sup> B cells in A.B. (2.3%) as compared to psoriasis with  $11.1 \pm 0.7\%$  and healthy controls with  $9.4 \pm 0.8\%$  (Fig. 3e).

In contrast to the above-described cohort of patients with psoriasis and healthy controls, A.B. showed increased



**Figure 3** Index patient A.B. suffering concomitantly from psoriasis and a common variable immune deficiency (CVID). Patient A.B. displayed the full phenotype of psoriasis vulgaris with demarcated red scaly plaques all over the body (a). Histological examination showed characteristic epidermal thickening with regular elongation of rete ridges, lymphocytic infiltration and neutrophilic micro-abscesses (b). RNA sequencing of lesional skin and analysis of genetic variants in A.B. revealed six synonymous and one non-synonymous variants in three of the 13 described CVID genes, namely NFKB1, NFKB2 and PRKCD, and one non-synonymous variant in TNFSF13, the ligand of the TNFRSF13B receptor (c). In A.B. IgA levels are markedly reduced as compared to psoriasis and healthy controls (d). Also, B-cell levels are decreased as compared to psoriasis and healthy controls (e). Moreover, A.B. showed increased percentages of new memory CD19<sup>+</sup>CD38<sup>-</sup>CD24<sup>-</sup> B cells, primarily memory CD19<sup>+</sup>CD38<sup>-</sup>CD24<sup>hi</sup> B cells and CD19<sup>+</sup>CD38<sup>hi</sup>CD24<sup>-</sup>CD138<sup>hi</sup> plasma cells, whereas the population of CD19<sup>+</sup>CD38<sup>int</sup>CD24<sup>int</sup> mature B cells (16%) and CD19<sup>+</sup>CD38<sup>hi</sup>CD24<sup>hi</sup> regulatory B cell was markedly decreased or almost absent (e).

percentages of new memory CD19<sup>+</sup>CD38<sup>-</sup>CD24<sup>-</sup> B cells (13.7%), primarily memory CD19<sup>+</sup>CD38<sup>-</sup>CD24<sup>hi</sup> B cells (63.4%) and CD19<sup>+</sup>CD38<sup>hi</sup>CD24<sup>-</sup>CD138<sup>hi</sup> plasma cells (51.6%), whereas the population of CD19<sup>+</sup>CD38<sup>int</sup>CD24<sup>int</sup> mature B cells (16%) and CD19<sup>+</sup>CD38<sup>hi</sup>CD24<sup>hi</sup> regulatory B cell was markedly decreased or almost absent (0.16%).

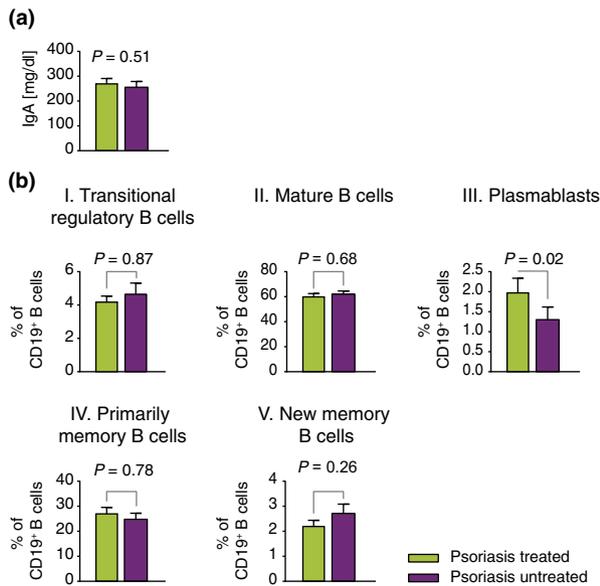
**B-cell subsets in patients under disease-controlling systemic antipsoriasis treatment regimens remain broadly unchanged**

To further understand the relevance of B cells in the context of psoriasis, we analysed IgA levels and above characterized B-cell subsets in a group of psoriasis patients showing stable disease activity (PASI < 5 in all patients) under systemic treatment. For IgA levels, no significant differences between the cohort of untreated patients (PASI > 5) and treated patients (PASI < 5) were detected (P = 0.5148, Fig. 4a). Moreover, PASI did not correlate with IgA (Figure S3D, Supporting Information) within the cohort of treated patients. Apart from significant reduced percentages of plasmablasts (CD19<sup>+</sup>CD24<sup>-</sup>CD38<sup>+</sup>) within the group of treated patients as compared to the group of untreated

patients (P = 0.02, Fig. 4b), no differences between treated and untreated patients were observed within the remaining four subsets of new memory B cells, transitional regulatory B cells, primarily memory B cells and mature B cells (Fig. 4b). Also, when comparing CD19<sup>+</sup>CD24<sup>-</sup>CD38<sup>+</sup>CD138<sup>+</sup> plasma cells between the two cohorts, we found no significant difference between treated and untreated patients (P = 0.14, Figure S3A, Supporting Information). Besides, the CD19<sup>+</sup>CD24<sup>-</sup>CD38<sup>+</sup>CD138<sup>+</sup> plasma cells – for which we showed positive correlations with IgA and PASI in the untreated cohort (Fig. 1b) – did not correlate with IgA and PASI within the group of treated patients (Figure S3B, C, Supporting Information).

**Discussion**

Psoriasis is understood as a T-cell-driven chronic inflammatory skin disease. In contrast to T-cell research in the field of psoriasis, B cells as important counterparts of T cells in the adaptive immune system have not yet been investigated comprehensively. Several case reports described new-onset psoriasis during B-cell-depleting therapies.<sup>21–24</sup> Moreover, studies investigating B-cell functions and phenotypes in psoriasis have revealed



**Figure 4** B-cell subsets in patients under disease-controlling systemic antipsoriasis treatment regimens remain broadly unchanged. For IgA levels, no significant differences between the cohort of untreated psoriasis patients (PASI > 5) and psoriasis patients under disease-controlling systemic treatment (PASI < 5) were detected (a). Apart from significant reduced percentages of plasmablasts (CD19<sup>+</sup>CD24<sup>-</sup>CD38<sup>+</sup>) within the group of treated patients as compared to the group of untreated patients ( $P = 0.02$ , b), no differences between treated and untreated patients were observed within the remaining four subsets of new memory B cells, transitional regulatory B cells, primarily memory B cells and mature B cells (b).

contradictory results. A study investigating natural killer cells and B cells in psoriasis found increased levels of B cells in psoriasis as compared to healthy controls,<sup>14</sup> whereas Czarnowicki *et al.*<sup>18</sup> found similar frequencies of B cells in psoriasis and healthy controls – a finding which is in line with our results. While we did not detect differences in the frequency of CD19<sup>+</sup>CD38<sup>hi</sup>CD24<sup>hi</sup> regulatory B cells, one study showed increased frequencies<sup>25</sup> and another one even decreased frequencies of these regulatory B cells in psoriasis.<sup>18</sup>

In our study, we found increased IgA levels in treatment-naïve patients with psoriasis in the serum corroborating the findings of earlier studies.<sup>26,27</sup> Besides, we found correlation of IgA levels with CD24<sup>-</sup>CD38<sup>+</sup>CD138<sup>+</sup> plasma cells in our cohort of untreated patients. The relative distribution of this subset was not different between the cohort of psoriasis and healthy volunteers; however, we found a significant correlation of this subset in untreated patients with PASI. This indicates that these cells might exert a distinct specific role in the inflammatory processes of psoriasis. However, during disease-controlling systemic treatment, neither did IgA levels and percentages of

CD19<sup>+</sup>CD24<sup>-</sup>CD38<sup>+</sup>CD138<sup>+</sup> plasma cells drop, nor did CD138<sup>+</sup> plasma cells correlate with PASI and IgA levels indicating a rather epiphenomenal than a disease-influencing role of IgA and CD19<sup>+</sup>CD24<sup>-</sup>CD38<sup>+</sup>CD138<sup>+</sup> plasma cells. Along with this, we only found IgA deposits in the dermal blood vessels, but not in the tissue as in dermatitis herpetiformis where it plays a clear pathophysiological role.<sup>28</sup> IgA in the dermal vessels of skin sections has been described for Henoch–Schönlein purpura<sup>29</sup> but also for apparently healthy skin of patients with primary IgA nephropathy, Henoch–Schönlein purpura or alcoholic liver disease.<sup>30</sup> These diseases including psoriasis are characterized by generalized inflammation, leading to the assumption that IgA in blood vessels but not in the skin might be an epiphenomenal finding in the course of generalized inflammation.

Furthermore, we analysed a patient (A.B.) suffering from psoriasis and B-cell deficiency concomitantly, thus representing an ideal role model to investigate the significance of B cells as a proinflammatory player in the pathogenesis of psoriasis in humans. On the transcriptome level, we found six synonymous and one non-synonymous variants in three of the 13 CVID genes NFKB1, NFKB2 and PRKCD. Besides, we identified a non-synonymous variant in TNFSF13, a ligand of the TNFRSF13B receptor. This receptor belongs to one of the 13 described CVID genes and has been shown to be involved in B-cell function and – in its mutated form – to disrupt antibody production leading to immune dysfunction.<sup>31</sup> As shown by our network analysis, TNFSF13 and its receptor show strong integration with other factors of the CVID network. As expected by the genetic background, our patient presented with decreased B-cell counts and IgA level, but high disease score, thus indicating that B cells and IgA deposits might be dispensable for the development of psoriasis. As psoriasis patients did not show an overall decreased frequency of CD24<sup>hi</sup>CD38<sup>hi</sup> regulatory B cells, a diminished protective B-cell phenotype as a general mechanism in the pathogenesis of psoriasis can be excluded. However, a decreased protective role of, e.g., regulatory B cells influencing the pathogenesis in a subset of patients cannot be excluded by our data.

As the functions of B cells are not limited to the production of antibodies, but also include activation of T cells, we also investigated T-cell function in our patient A.B. We showed that T cells are fully functional in A.B. with a psoriasis typical Th17 phenotype.

In A.B. rather low frequencies of regulatory B cells were detected, a common finding in patients with common variable immunodeficiency.<sup>32</sup> As regulatory B cells have been shown to play an important role in the development of regulatory T cells and for controlling T-cell activation and thus autoimmunity,<sup>32</sup> one might argue that A.B. developed psoriasis due to limited regulatory B-cell-mediated control of T-cell activity. However, in our cohort of psoriasis patients an overall decreased frequency of CD24<sup>hi</sup>CD38<sup>hi</sup> regulatory B cells could not be

detected strongly questioning a general mechanism of protective B-cell phenotypes in the pathogenesis of psoriasis. Along with this, Yanaba *et al.* showed that in the murine imiquimod model, skin inflammation was not less but in the contrary more severe in CD19<sup>-/-</sup> mice than in WT mice corroborating our point of CD19<sup>+</sup> cells not primarily driving psoriatic inflammation. Yet, they found that inflammatory responses were negatively regulated by a unique IL-10-producing CD1d<sup>hi</sup>CD5<sup>+</sup> regulatory B-cell subset<sup>33</sup> hinting at a decreased protective role of regulatory B cells in a subset of psoriasis patients, e.g., in our patient A.B. However, in A.B. the positive family history of psoriasis but not COVID challenges a crucial role of B cells. Consistent with this genetic line of argumentation, microarray data, including ours, as well as big GWAS have mainly found aberrations in T-cell and not B-cell-related pathways.<sup>34–36</sup> Not least our immunofluorescence stainings revealed that B cells are rarely found in psoriatic skin as compared to the dense infiltrate of T cells providing one reason why B-cell-depleting therapies have not proven resounding success for the therapy of psoriasis in contrast to the successful target-oriented therapies that are focused on T-cell-mediated pathways.<sup>37</sup>

Although we analysed a broad set of surface markers used for in-depth phenotyping of B cells, we eventually found rather few differences between the B-cell subtypes of psoriasis as compared to healthy volunteers. This hints at the conclusion that psoriasis can be elicited in the lack of B cells and immunoglobulins. Nevertheless, it might be possible that relevant changes in B-cell subsets are not detectable in the periphery, but in primary and secondary lymphoid organs such as bone marrow or lymph nodes, which were not accessible in our study. Although reduced frequencies of regulatory and potential protective B-cell subsets as a general pathomechanism for psoriasis could be excluded based on our data, they might be relevant for a subset of patients. Further functional studies beyond pure characterization of surface markers – even if as detailed as in our study – are necessary to reveal the disease-specific contribution of B cells and thus the therapeutic potential of targeting specific B-cell subtypes.

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## Supporting information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** C3 and C4 complement was higher in the serum of psoriasis than in healthy controls (A and B)

**Figure S2.** Peripheral blood mononuclear cells (PBMC) of A.B. showed substantial proliferation when stimulated by  $\alpha$ -CD3 and  $\alpha$ -CD28, Phytohemagglutinin (PHA) and pertussis toxin as assessed by H3-Thymidine incorporation (A)

**Figure S3.** Regarding the subset of CD19<sup>+</sup>CD24<sup>-</sup>CD38<sup>+</sup>CD138<sup>+</sup> plasma cells no significant difference between treated and untreated patients were found (A)

**Figure S4.** Representative immunofluorescence staining of psoriatic skin in two patients shows that CD19<sup>+</sup> cells are not (A) or rather rarely (B) found in psoriatic skin as compared to CD8 positive T cells

**Figure S5.** Representative gating strategy for IgA<sup>+</sup> plasma cells (A): After gating on lymphocytes, exclusion of doublets and and gating on live cells, B cells were determined by CD19 expression